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ANTIOXIDATIVE ENZYME AND PHYTOCHEMICAL RESPONSE OF RICE CULTIVARS (*ORYZA SATIVA*. L) TO RICE BLAST DISEASE *(MAGNAPORTHE ORYZAE*)

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ABSTRACT

Rice blast is one of the major diseases in rice growing area which is caused by Magnaporthe oryzae causing approximately 80% yield loss. The present investigation was performed to elucidate the role of plant antioxidant enzymes such as catalase, superoxide dismutase, peroxidase, glutathione reductase, phenylalanine ammonialyase, and nitrogen assimilatory enzymes like nitrate and nitrite reductase in imparting the resistance/susceptibility to rice blast infection in two rice genotypes (BR-2655 and HR-12). Sowing was done in pots and maintained in green house. Artificial inoculation was done after 45 days of sowing [DAS], control and inoculated leaves were collected after 48 hours of inoculation and antioxidant enzyme activity and changes in lignin content were analyzed. Fungal pathogen inoculation (M. oryzae) induced significantly higher level of antioxidative enzymes catalase (31.84%), superoxide dismutase (35.88%), peroxidase (48.57%), glutathione reductase (27.38%) PAL (152.2%), activity in resistant genotype (BR-2655) than susceptible genotype (HR-12). There was also hike in the lignin content in resistant genotype compared to susceptible. Increase in the PAL and POX content leads to high production of phenolic compounds, lignin content and antifungal role by cell wall strengthening to enhance resistance against pathogen. The results suggested that enhanced activities of enzymes and high lignin content may contribute resistance in rice plants against rice blast infection.

KEYWORDS: Rice Blast, Magnaporthe Oryzae & Antioxidative Enzymes

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Original Article

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important cereal crops, providing a staple food for nearly half of the global population. Grain yield has doubled since 1960 because of prevalent cultivation of hybrid and semi-dwarf rice varieties [9].

Conversely, rice production is continually threatened by disease, insects and other stress. The various diseases caused by fungi, bacteria, viruses and nematodes have been accounted on rice [27]. Rice blast is one among them. This is the most destructive fungal disease, which largely widespread in rice growing fields causing significant reduction in grain quality and yield [5]. The severe yield loss up to 85% has been reported because of rice blast disease alone in worldwide [4].

The blast fungus infects the rice plants in all the growth stages and symptoms of the disease can be observed in aerial parts. The disease symptoms appear as white to grey or brown spindle-shaped lesions or leaf spots followed by nodal and neck blast, later which can causes necrosis and finally death of the host plant. Most infections occur on the leaves during vegetative stage and on panicle and neck during reproductive stage of the crop [5].

When plant attacked by any kind of pathogen, the instant reactions elicited by the plant is an oxidative burst which is one of the most rapid defense reactions leads to the transient production of high levels of reactive oxygen species (ROS) that include superoxide (O_{2^-}), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH•). To avoid latent damage caused by reactive oxygen species (ROS) to cellular components and for scavenging the excess ROS, plants can activate well-organized enzymatic and non-enzymatic antioxidant cascade for maintaining equilibrium between the production and detoxification of ROS [15]. The enzymatic protective mechanism operates by sequential and simultaneous activation of several antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), glutathione reductase (GR) and polyphenol oxidase (PPO). Non-enzymatic components include secondary metabolites such as tocopherol, carotenoids, chitin, lignin and phenolic compounds [15]. Maintenance of high antioxidant capability to scavenge the toxic ROS is essential to increase the environmental stress tolerance in plants [3].

In this present investigation defense related enzymes such as superoxide dismutase, catalase, peroxidase, phenyl ammonia lyase, activity nitrogen assimilating enzymes like nitrate reductase and nitrite reductase activity were analyzed for better understanding of resistance mechanism involved against rice blast pathogen (*Magnaporthe oryzae*) in two rice genotypes.

MATERIALS AND METHODS

Plant Material and Experimental Design

Two rice cultivars of rice (*Oryza sativa* L.) BR-2655 (blast resistant) and HR-12 (blast susceptible) were chosen to study the biochemical response of antioxidant enzymes and nitrate assimilatory enzymes. Each cultivar was evaluated by pot culture experiments in polyhouse under two treatments control and inoculated. After 45 days of sowing the artificial inoculation of *Magnaporthe oryzae,* pure culture was done by spraying to ensure the maximum disease pressure. The leaves samples were collected after 48 hours of infection for determining the antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), Nitrate (NR) and Nitrite reductase (NiR).

Extraction of Enzyme and Determination of Protein Content

Fresh leaf tissues after collection were processed immediately for enzyme extraction between 0°C and 4°C) and used for the assay. In order to measure the enzyme activities of SOD, CAT, GR, NR and NiR, 0.5 g of healthy and infected leaf tissues were taken and ground into fine powder with liquid nitrogen and extracted with 2 ml of 0.05M sodium phosphate buffer of pH 7.8 and pH 7.0 for SOD and CAT respectively. POX was extracted in 0.1 M potassium phosphate buffer (pH 7.0). Grinding buffer for GR included 0.1M Tris–HCl pH 7.8 and 2mM dithiothreitol (DTT). PAL was extracted in 0.1 M Tris buffer (pH 8.5). Nitrite reductase (NiR) enzyme extract was prepared in 0.1 M phosphate buffer, pH 7.5, containing 10 mM cysteine. Then homogenate was centrifuged at 14,000 rpm for 20 min at 4°C, the supernatant was used as enzyme source for assay. Protein content was estimated using Lowry's method.

ANTIOXIDANT ENZYME ASSAYS

Catalase

CAT (EC 1.11.1.6) activity in rice leaves was spectrophotometrically determined by Beers and Sizers method [1]. The reaction mixture contained 2.98 mL of 16.65 mM hydrogen peroxide in 50 mM phosphate buffer, pH 7.0 and 20 µL of enzyme extract was used to initiate the reaction. The decrease in absorbance at 240 nm was measured for 5 minutes using the substrate blank. One unit of CAT is defined as the one µmole of H_2O_2 decomposed per minute at pH 7.0 at 25°C and specific activity was expressed as µmole min^{-1} mg^{-1} protein.

Superoxide Dismutase

The activity of SOD (EC 1.15.1.1) in rice leaves was assayed photochemically at 560 nm by the Beauchamp and Fridovich method [2]. About 3 mL of reaction mixture contained 20 µL of enzyme extract, 10 mM of L-methionine, 33 µM of p-nitrobluetetrazolium chloride, 066 µM of ethylenediaminetetraacetic acid (EDTA), 3.3 µM of riboflavin in a 50 mM potassium phosphate buffer of pH 7.8. The assay was initiated by adding riboflavin and took place in a glass tube illuminated by a 15 W fluorescent lamp at 25°C for 20 minutes. The increase in absorbance of the blue formazan produced by NBT photo reduction was measured at 560 nm. A blank was maintained with all the constituents but in the dark. One unit of SOD is defined as the amount of enzyme required to inhibit 50% of the NBT photo-reduction per minute and specific activity is expressed as IU per mg protein.

Peroxidase Activity

The peroxidase (POX, EC 1.11.1.7) activity in rice leaves was spectrophotometrically determined [8]. Twenty microliter of the enzyme extract was added to the reaction mixture consisting 2.88 mL of 0.1 M potassium phosphate buffer (pH 7.0), 50 µL of 0.02 M guaiacol, 50 µL of 0.042% H_2O_2 and increase in optical density was measured at 436 nm for 5 minutes. One unit of POX is defined as the amount of enzyme which catalyses the formation of one micromole of oxidized guaiacol per minute at 25°C and the specific activity was expressed as µmole $min^{-}mg^{-1}$ protein.

Glutathione Reductase

GR (EC 1.8.1.7) activity in rice leaves was determined spectrophotometrically by Mavis and Stellwagen method [20]. The reaction mixture contained 100 µL of 30 mM oxidized glutathione, 1.5 mL of 100 mM potassium phosphate buffer with 3.4 mM EDTA, pH 7.6, 350 µL of 0.8 mM ß-nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) and 950 µL of water. The decrease in absorbance at 340 nm on addition of 100 µL of enzyme to reaction mixture was recorded for 5 minutes. One GR unit is defined as the amount of enzyme that oxidises 1.0 µmole of NADPH per minute at pH 7.6 at 25°C and specific activity is expressed as µmole min^{-1} mg^{-1} protein.

Phenylalanine Ammonia Lyase

The PAL (EC.4.3.1.5.) activity in rice leaves was determined spectrophotmetrically by the method of Paltonen and Karjalainen [17]. Assay mixture included 0.5 ml of enzyme extract and 2.5 mL of 0.2% L-phenylalanine in 0.1 M Tris buffer (pH 8.5) which was incubated at 40 °C for 1 hr. The reaction was stopped by 0.2 M HCl and the absorbance was measured at 290 nm at periodic 5 min intervals for 30 minutes against substrate blank. One unit of PAL is defined as the amount of enzyme required to liberate 1 micromole of transcinnamte from L-Phenylalanine per minute and the specific activity was expressed as µmol/min/mg protein.

Nitrate Reductase Assay

NR (EC 1.7.1.1) activity in rice leaves was spectrophotometrically determined by the method of Hageman and Reed [7]. A known weight (0.5 g) of fresh tissue was cut into pieces and suspended in screw cap vials containing 3.5 ml of incubation mixture (20 ml of 0.1 M phosphate buffer, 20 ml of 5 per cent propanol and 10 ml of 0.2 per cent KNO_3). The vials were sealed and kept in dark condition at 30°C for 2 h. Nitrite released in the medium was determined by treating 1 ml aliquot with 1 ml each of 1% sulphanyl amide and 0.02% N-1-napthyl ethylene diamine hydrochloride. After 20 min, solution is diluted to 5 ml with water and absorbance is measured at 540 nm. Standard curve is prepared by using reagent grade

concentrations of nitrite (KNO_2) solution. The nitrate reductase activity is expressed as n moles of nitrite formed per hour per gram fresh weight.

Nitrite Reductase Assay

NiR (EC 1.7.7.1) activity in rice leaves was determined spectro photo metrically by the method of Wray and Fido [24]. The 0.8 ml of reaction mixture contained 0.2 ml of 0.1M phosphate buffer, 0.1 ml of 5mM sodium nitrite, 0.1ml of 1.5 mM methyl viologen, 50 µl of enzyme and distilled water. The reaction was started by adding 0.2 ml of the 2.5% sodium dithionite reagent and incubated for 10 minutes. The reaction was stopped by vigorously shaking the mixture until the dithionite was completely oxidized and the dye colour disappeared. For determination of nitrite consumed by enzyme, 50 µl aliquot of above mixture was made to 2.0 ml using distilled water and 1.0 ml of 1% sulphanilamide followed by 1 ml of 0.02% NNED was added and incubated for 15 minutes. Blank was also processed in the similar way except for 50 µl of enzyme was added after the addition of sulphanilamide and NNED and read at 540 nm. The nitrite consumed by the action of enzyme was estimated from the nitrite standard curve. NiR activity is expressed as µmol of nitrite consumed/min and the specific activity as enzyme activity/mg protein.

Determination of Lignin Content in Rice Leaves Due to *Magnaporthe Oryzae* Infection

Lignin: Lignin content in leaf tissue was determined by acetyl bromide method. The values obtained were expressed as g per 100 gram dry weight.

Statistical Analysis

The experimental data was analyzed statistically following the method described by Gomez and Gomez [6]. The results were expressed as mean and standard error of mean of five replicates of the biochemical parameter for each sample. $p<0.05$ was used as significance level for "F" and "T" test. 2 factorial completely randomized design was used to calculate critical difference where ever "F" was found to be significant.

RESULTS

Changes in Activities of Anti-Oxidant Enzymes In Leaves of Rice Cultivars Due to *Magnaporthe Oryzae* Infection.

Catalase

The activity of Catalase (Table 1) in the leaves of rice genotype was significantly differed in control and 48 hr after inoculation. Catalase activity in all the genotypes were increased significantly in inoculated leaves (27.01U/ mg protein) as compared to control (20.46 U/ mg protein). Amongst all the genotypes the catalase activity in the resistant genotype BR-2655 was significantly higher (25.78 U/ mg protein) when compared to susceptible genotype (22.08 U/mg protein). In case of treatment, inoculated leaves of resistant genotype BR-2655 recorded high Catalase activity (29.34 U/mg protein) as compared to control leaves (21.44U/mg protein). Inoculated leaves of susceptible HR-12 genotype showed least catalase activity (24.68 U/mg protein) as compared to control leaves (19.48 U/mg protein). Compared to all genotypes, inoculated leaves of BR-2566 showed highest increase in catalase activity (31.84%) where as HR-12 recorded least increase (26.7%)**.**

Superoxide Dismutase (SOD)

The activity of superoxide dismutase (Table 2) in the leaves of rice genotype was significantly differed in control and 48 hr after inoculation. Superoxide dismutase activities in all the genotypes were increased significantly in

inoculated leaves (23.77 U/mg protein) as compared to control (18.64 U/mg protein). Amongst all the genotypes the superoxide dismutase activity in the resistant genotype BR-2655 was significantly higher (22.18 U/mg protein) when compared to susceptible genotype (20.23 U/mg protein). In case of treatment, inoculated leaves of resistant genotype BR-2566 recorded highest superoxide dismutase activity (25.56 U/mg protein) as compared to control leaves (18.81U/mg protein). Inoculated leaves of susceptible HR-12 genotype showed least superoxide dismutase activity (21.99 U/mg protein) as compared to control leaves (18.48 U/mg protein). Compared to all genotypes, inoculated leaves of BR-2655 showed highest increase in superoxide dismutase activity (35.88%) where as HR-12 recorded least increase (19 %)**.**

Peroxidase (POX)

The activity of peroxidase (Table 3) in the leaves of rice genotype was significantly differed in control and 48 hr after inoculation. Peroxidase activities in all the genotypes were increased significantly in inoculated leaves (1.865 U/mg protein) as compared to control (1.275 U/mg protein). Amongst all the genotypes, the peroxidase activity in the resistant genotype BR-2655 was significantly higher (1.74 U/mg protein) when compared to susceptible genotype (1.4 U/mg protein). In case of treatment, inoculated leaves of resistant genotype BR-2655 recorded high peroxidase activity (2.08 U/mg protein) as compared to control leaves (1.4 U/mg protein). Inoculated leaves of susceptible HR-12 genotype showed least peroxidase activity (1.65 U/mg protein) as compared to control leaves (1.15 U/mg protein). Compared to all genotypes inoculated leaves of BR-2655 showed highest increase in peroxidase activity (48.57%) where as HR-12 recorded least increase (43.47%)**.**

Glutathione Reductase (GR)

The activity of glutathione reductase (Table 4) in the leaves of rice genotypes was significantly differed in control and 48 hr after inoculation. Glutathione reductase activity in all the genotypes were increased significantly in inoculated leaves (0.875 U/mg protien) as compared to control (0.695 U/mg protein). Amongst all the genotypes the glutathione reductase activity in the resistant genotype BR-2655 was significantly higher (0.955 U/mg protein) when compared to susceptible genotype (0.615 U/mg protein). In case of treatment, inoculated leaves of resistant genotype BR-2655 recorded high glutathione reductase activity (1.07 U/mg protein) as compared to control leaves (0.84 U/mg protein). Inoculated leaves of susceptible HR-12 genotype showed least glutathione reductase activity (0.68 U/ mg protein) as compared to control leaves (0.55 U/mg protein). Compared to all genotypes inoculated leaves of BR-2655 showed highest increase in glutathione reductase activity (27.38%) where as HR-12 recorded least increase (23.63%)**.**

Phenylalanine Ammonialyase (PAL)

The activity of phenylalanine ammonialyase (Table 5) in the leaves of rice genotype significantly differed in control and 48 hr after inoculation. Phenylalanine ammonialyase activities in all the genotypes were increased significantly in inoculated leaves (2.74 U/mg protein) as compared to control (1.125 U/mg protein). Amongst all the genotypes the phenylalanine ammonialyase activity in the resistant genotype BR-2655 was significantly higher (2.4 U/mg protein) when compared to susceptible genotype (1.6 U/mg protein). In case of treatment, inoculated leaves of resistant genotype BR-2655 recorded high phenylalanine ammonialyase activity (3.43 U/mg protein) as compared to control leaves (1.36 U/mg protein). Inoculated leaves of susceptible HR-12 genotype showed least phenylalanine ammonialyase activity (2.05 U/mg protein) as compared to control leaves (1.14 U/mg protein). Compared to all

genotypes inoculated leaves of BR-2655 showed highest increase in phenylalanine ammonialyase activity (152.2%) where as HR-12 recorded least increase (79.8%)**.**

Changes in Activities of Nitrogen Assimilatory Enzymes in Leaves of Rice Cultivars due to Rice Blast Infection.

Nitrate Reductase

Nitrate reductase (Table 6) activity in the leaves of rice genotypes was significantly differed in control and 48 hr after inoculation. Levels of nitrate reductase activity in all the genotypes were significantly lower in inoculated leaves (0.74 U/mg protein) as compared to control (1.58 U/mg protein). Amongst all the genotype the nitrate reductase activity in the resistant BR-2655 genotypes was significantly higher (1.47 U/mg protein) when compared to susceptible HR-12 (0.845 U/mg protein). In case of treatment, inoculated leaves of resistant genotype BR-2655 recorded less difference in nitrate reductase activity (0.93 U/mg protein) as compared to control leaves (2.01 U/mg protein). Inoculated leaves of susceptible genotype HR-12 showed significant difference in nitrate reductase activity (0.55 U/mg protein) as compared to control leaves (1.15 U/mg protein). Compared to all genotypes, inoculated leaves of BR-2655 showed highest increase in nitrate reductase activity (53.73%) where as HR-12 recorded least increase (51.75%)**.**

Nitrite Reductase

Nitrite reductase (Table 7) activity in the leaves of rice genotypes significantly was differed in control and 48 hr after inoculation. Levels of nitrite reducatse activity in all the genotypes were significantly lower in inoculated leaves (0.355 U/mg protein) as compared to control (0.715 U/ mg protein). Amongst all the genotype the nitrite reductase activity in the resistant BR-2655 genotypes was significantly higher (0.69 U/mg protein) when compared to susceptible HR-12 (0.38 U/mg protein). In case of treatment, inoculated leaves of resistant genotype BR-2655 recorded less difference in nitrite reductase activity (0.44 U/mg protein) as compared to control leaves (0.94U/mg protein). Inoculated leaves of susceptible genotype HR-12 showed significant difference in nitrite reductase activity (0.27 U/mg protein) as compared to control leaves (0.49 U/mg protein). Compared to all, genotypes inoculated leaves of BR-2655 showed highest increase in nitrite reductase activity (53.19%) where as HR-12 recorded least increase (44.9%)**.**

Changes in Lignin Content in Leaves of Rice Cultivars due to *Magnaporthe Oryzae* Infection.

Lignin

Lignin content (Table 8) in the leaves of rice genotypes was differed significantly in control 48 hr after inoculation. Levels of lignin content in all the genotypes were significantly higher in inoculated leaves (7.41 g % dry wt) as compared to control (3.92 g % dry wt). Amongst all the genotype the lignin in the resistant genotype BR-2655 was significantly higher (7.6 g % dry wt) when compared to susceptible genotype HR-12 (3.71 g % dry wt). In case of treatment, inoculated leaves of resistant genotype BR-2655 recorded highest lignin content (10.33 g % dry wt) as compared to control leaves (4.91 g % dry wt). Inoculated leaves of susceptible genotype HR-12 showed least lignin content (4.49 g % dry wt) as compared to control leaves (2.93 g % dry wt). Compared to all, the genotype inoculated leaves of BR-2655 showed highest increase in lignin content (110.38 %) where as HR-12 recorded least increase (53.24%)**.**

DISCUSSIONS

Changes in Activities if Antioxidant Enzymes in Leaves of Rice Cultivars to Rice Blast Disease (*Magnaporthe Oryzae*).

Catalase

Catalase is an iron containing enzyme, which is responsible for catalyzing dismutation of hydrogen peroxide into water and molecular oxygen. Hydrogen peroxide H_2O_2 is mainly produced by photoresipiration, beta oxidation, etc. According to the results obtained (Table _) among the genotypes, BR-2655 recorded the highest CAT activity in rice leaves. Rice blast induced an increase in catalase activity in leaves of both genotypes, implying an efficient detoxification o f H_2O_2 and thereby contributes to the ROS tolerance of the species. The increased induced CAT activity in inoculated leaves of BR-2655 may be related to increased tolerance of the genotype to oxidative stress [25]; thus, exhibiting a more efficient tolerance mechanism during blast infection, by maintaining H_2O_2 homeostasis.

Superoxide Dismutase

Superoxide dismutase (SOD), one of the most important antioxidant enzymes plays a major role in scavenging of reactive oxygen species (ROS), it constitutes the first line of defense against ROS by catalyzing the dismutation of superoxide anion (O_2^-) to molecular oxygen and hydrogen peroxide (H_2O_2). In our present study, the SOD activity was increased in the inoculated leaves of BR-2655 variety compared to control and susceptible variety of HR-12. SOD activity in the leaves in BR-2655 genotype was higher in control compared to susceptible genotypes and increased (35.88%) under inoculation. Susceptible genotypes HR-12 showed low SOD activity in control and significantly increased (19%) upon *Magnaporthe oryzae* culture inoculation

Peroxidase

Peroxidases are heme containing enzymes which primarily use hydrogen peroxide as electron donar later which use aromatic electron donar like guaiacol, ascorbate and pyragallol. For many POX enzymes hydrogen peroxide is an optimal susbtrate but some are used other organic substrates such as ascorbate peroxide, lipid peroxide, etc. As per the result obtained by (Table 3) POX activity in the leaves in BR-2655 genotype was higher in control compared to susceptible genotypes and increased (48.58%) under inoculation. In our study, less POX activity was observed in comparison with CAT and SOD. Generally, CAT and POX enzymes catalyzes same reaction with the exception that peroxidase need electron donor. The peroxidase activity was increased significantly along with highest lignin content. And they found both SOD and POX enzymes were associated with lignin formation which inturn leads to resistant mechanism to rice head and leaf blight. These results were observed in the study conducted by Taheri et al. [22] where rice plants are response to rice leaf and head blight caused by *Alternaria alternate*.

Glutathione Reductase (GR)

Glutathione reductase is another important antioxidant enzyme for scavenging excess of ROS produced by the cells result of stress. This mainly scavenges the singlet oxygen and hydrogen peroxide. Glutathione reductase is a flavo protein oxidoreductase which catalyses the reduction of gluthione disulfide (GSSG) to sulfhydryl form of glutathione (GSH). GR utilizes FAD as prosthetic group and NADPH as reductant to reduce GSSG to GSH. In this present investigation there is a slight increase in GR activity in inoculated leaves of BR-2655 variety as compared to control, where as HR-12 shows lease increase in both inoculated and control leaves. Thus, increase in catalase, superoxide

dismutase and glutathione reductase could be taken as support for the notion that the components of the ascorbate-glutathione cycle are co-regulated [21].

Phenylalanine Ammonia Lyase (PAL)

Phenylalanine ammonia lyase is most important enzyme in phenyl proponoid pathway, therefore, it involved in the biosynthesis of secondary metabolites like flavanoids, lignin and phenylproponoids using L-phenylalanine as a precursor in plants. PAL catalyses the deamination reaction converting L-phenylalanine to ammonia and transcinnamic acid. In present study, PAL activity in the leaves in BR-2655 genotypes was higher in control compared to susceptible genotypes and increased significantly (152.2%) under inoculation. Susceptible HR-12 show less PAL activity in control and increased significantly (79.8%) upon *Magnaporthe oryzae* culture inoculation. Increase in the PAL is directly involved in the biosynthesis of Salicylic acid (SA) and biosynthesis of several defense-related secondary metabolites such as lignin and phenols [16].

Nitrate Reductase (NR)

Nitrate reductase are molybdoenzymes that catalyses the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) which is subsequently reduced to ammonia by nitrite reductase enzyme which is the biological process of conversion of inorganic nitrate to ammonia. The primary function of NR in plants is nitrogen assimilation, and it is an important source of nitric acid (NO). The produced ammonia is then incorporated into amino acids and other nitrogen derived constituents through GS-GOGAT (glutamine synthetase-glutamine-2-oxoglutarate transminase) pathway. In the present study (Table 6), the NR showed decreased activity in leaf of both cultivars as compared to controls. Among genotypes BR-2655 maintained higher NR activity compared to susceptible HR-12. The study reveals that the rice blast infection may reduce the nitrogen assimilation and also less efficiency in conversion of nitrate to ammonia in rice cultivars.

Nitrite Reductase (NiR)

Nitrite reductase (NiR) The primary role of Nitrite reductase in plants is the reduction of nitrite to ammonia. The nitrite reduction takes place by enzymatic mechanisms that include the reaction catalyzed by nitrite reductase (NiR) in the cytosol [18, 26]. The susceptible variety HR-12 (Table 7) showed a decreased activity as compared to resistant variety BR-2566 where as it shows less reduction in NiR activity. This could be attributed to the fact that non-enzymatic nitrite reduction occurs spontaneously in the apoplast due to the acidic conditions or to the presence of ascorbic acid or phenols [37].

Changes in Lignin Content in Leaves of Rice Cultivars Due to *Magnaporthe Oryzae* Infection

Lignin

As per the result obtained by this investigation, among the genotypes the lignin content in inoculated leaves of resistant genotype BR-2655 increased maximum about 153% (Table 8) as compared to susceptible variety i.e., HR-12 whereas it shows least lignin content. This result revealed that when the blast disease induced in rice leaves which leads to higher production of lignin to provide defence against fungal pathogen. Lignin produced by the biological process called lignification in plant tissues which is inedible to pathogens. [10] Lignin protects the plant by cell wall mediated defence mechanism against pathogenic fungal penetration into plant. Hence, it is considered as first defence barrier against successful penetration of invasive pathogens. Lignin deposition during pathogen attack is well documented as a plant

defence response. After pathogen invades the plant, during plant defence response, lignin or lignin like phenolic compound accumulation was shown to occur in a variety of plant microbe interactions during the plant defence responses [23].

CONCLUSIONS

Overall study revealed that the higher amount of antioxidant enzymes and nitrogen assimilation enzymes activity play an important role in defense mechanism of rice plants against rice blast (*Magnaporthe oryzae*) infection in case of resistant varieties. These biochemical factors could be a potential tool that can be used for the selection of resistance lines against rice blast disease.

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Application of Research: The resistant cultivar BR-2655 will be used for further breeding program to come up with a better resistant variety. The information obtained from the present investigation will be utilized to design and conduct molecular experiments involving marker assisted selection, transcriptome analysis and stress response protein identification.

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Author Contributions:

Shwetha, M. D.: The graduate student has carried out the research work as a part of her M.Sc. Programme.

Kiran Kamalakar Mirajkar: Principle Investigator, who has conceptualized, designed the research programme and arranged for all the infrastructural facilities required for the research programme.

Virupaksha Prabhu, H.: Professor of Plant pathology has guided and helped us in conducting pot culture studies in poly house.

ABBREVIATIONS:

PAL: Phenylalanine ammonia Lyase

POX: Peroxidase

CAT: Catalase

SOD: Supreroxide Dismutase

GR: Glutathione Reductase

NR: Nitrate Reductase

NiR: Nitrite Reductase

NBT: p-nitrobluetetrazolium chloride

DTT: dithiothreitol

EDTA: ethylenediaminetetraacetic acid

NADPH: ß-nicotinamide adenine dinucleotide phosphate (reduced form)

NNED: N-1- napthyl ethylene diamine hydrochloride

DMSO: Dimethyl sulphoxide

Table 1: Activity of Catalase in Leaves of Different Rice Genotypes in Response to Rice Blast Infection

Genotype	Catalase (U/mg protein)			
	Control	Inoculated	% Increase	Mean
HR-12 (S)	19.48	24.68	26.7	22.08
BR-2655 (R)	21.44	29.34	31.84	25.78
Grand mean	20.46	27.01	29.27	23.93
	S.Em±		**CD @ 5%**	
Factor G	0.112		0.335	
Factor T	0.112		0.335	
G × T	0.158		0.474	

G: Genotype, T: Treatment, R: Resistant genotype, S: Susceptible genotype.

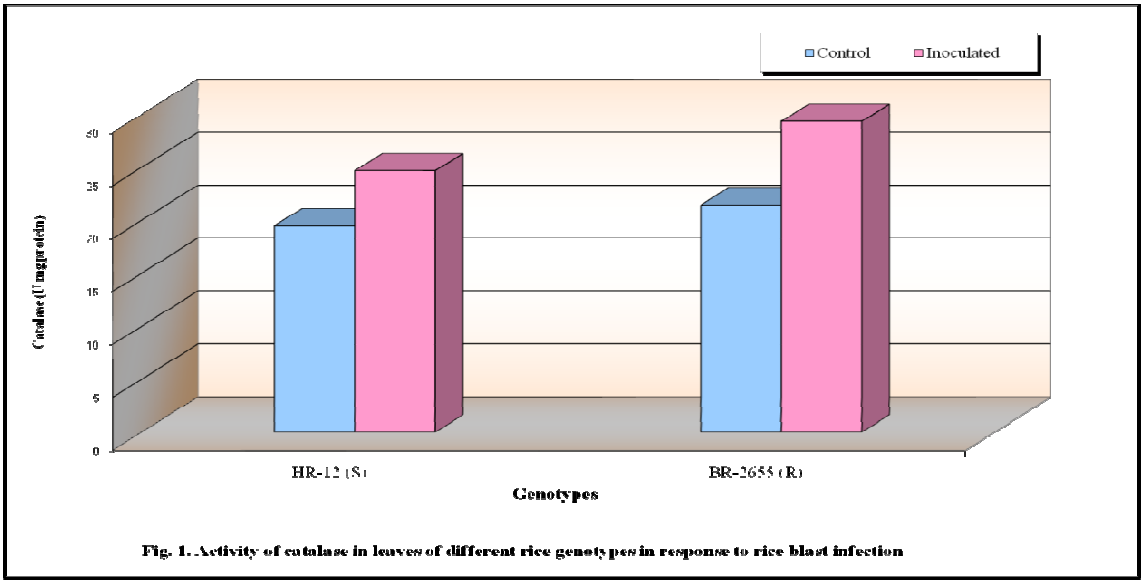

Figure 1: Activity of Catalase in Leaves of Different Rice Genotypes in Response to Rice Blast Infection.

Table 2: Activity of Superoxide Dismutase in Leaves of Different Rice Genotypes in Response to Rice Blast Infection

Genotype	Superoxide dismutase (U/mg protein)			
	Control	Inoculated	% Increase	Mean
HR-12 (S)	18.48	21.99	19	20.23
BR-2655 (R)	**18.81**	**25.56**	35.88	22.18
Grand mean	18.64	23.77	27.44	21.205
	S.Em±		**CD @ 5%**	
Factor G	0.016		0.48	
Factor T	0.016		0.48	
G × T	0.227		0.679	

G: Genotype, T: Treatment, R: Resistant Genotype, S: Susceptible Genotype.

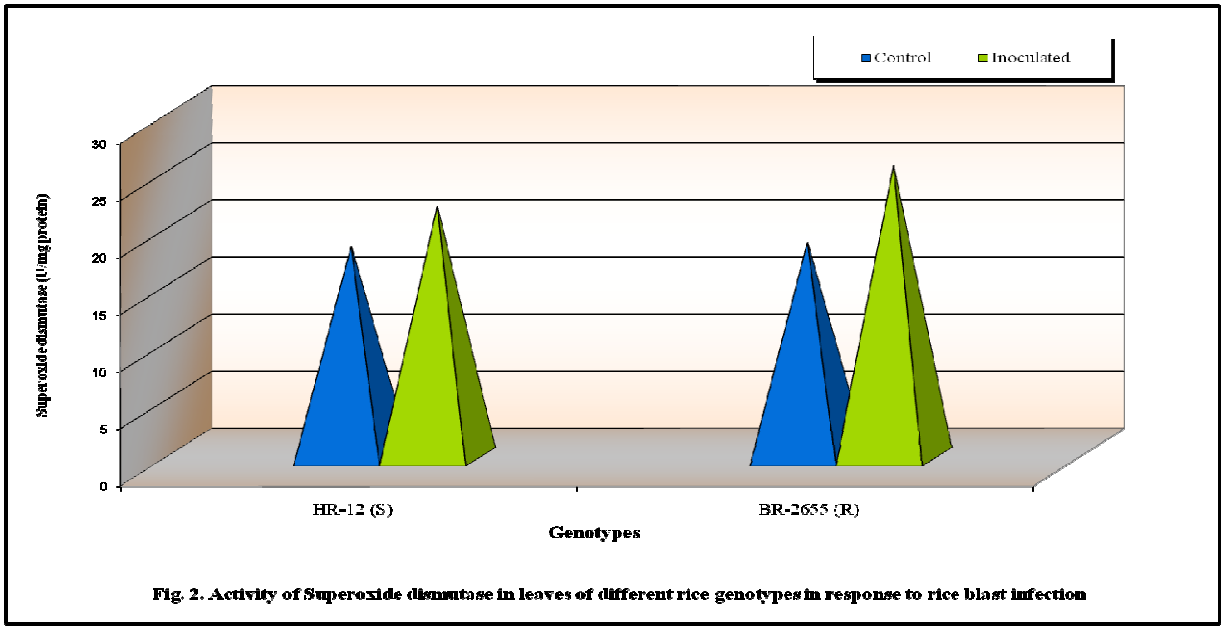

Figure-2 Activity of Superoxide Dismutase in Leaves of Different Rice Genotypes in Response to Rice Blast Infection.

Table 3: Activity of Peroxidase in Leaves of Different Rice Genotypes in Response to Rice Blast Infection

Genotype	Peroxidase (U/mg protein)			
	Control	Inoculated	% Increase	Mean
HR-12 (S)	1.15	1.65	43.47	1.40
BR-2655 (R)	**1.40**	**2.08**	48.57	1.74
Grand mean	1.27	1.86	46.02	1.57
	S.Em±		**CD @ 5%**	
Factor G	0.022		0.067	
Factor T	0.022		0.067	
G × T	0.032		0.095	

G: Genotype T: Treatment, R: Resistant Genotype, S: Susceptible Genotype.

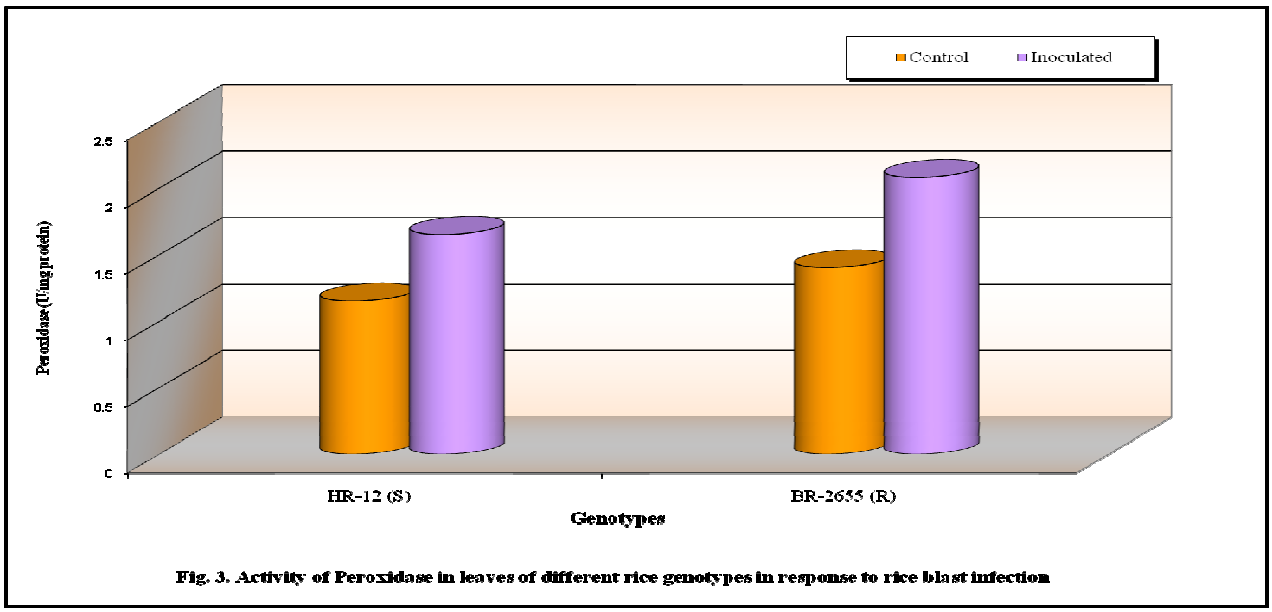

Figure: 3 Activity of Peroxidase in Leaves of Different Rice Genotypes in Response to Rice Blast Infection.

Table 4: Activity of Glutathione Reductase in Leaves of Different Rice Genotypes in Response to Rice Blast Infection

Genotype	Glutathion reductase (U/mg protein)			
	Control	Inoculated	% Increase	Mean
HR-12 (S)	0.55	0.68	23.63	0.61
BR-2655 (R)	**0.84**	**1.07**	27.38	0.95
Grand mean	0.69	0.87	25.50	0.78
	S.Em±		**CD @ 5%**	
Factor G	0.011		0.033	
Factor T	0.011		0.033	
G × T	0.016		0.047	

G: Genotype T: Treatment, R: Resistant genotype, S: Susceptible genotype

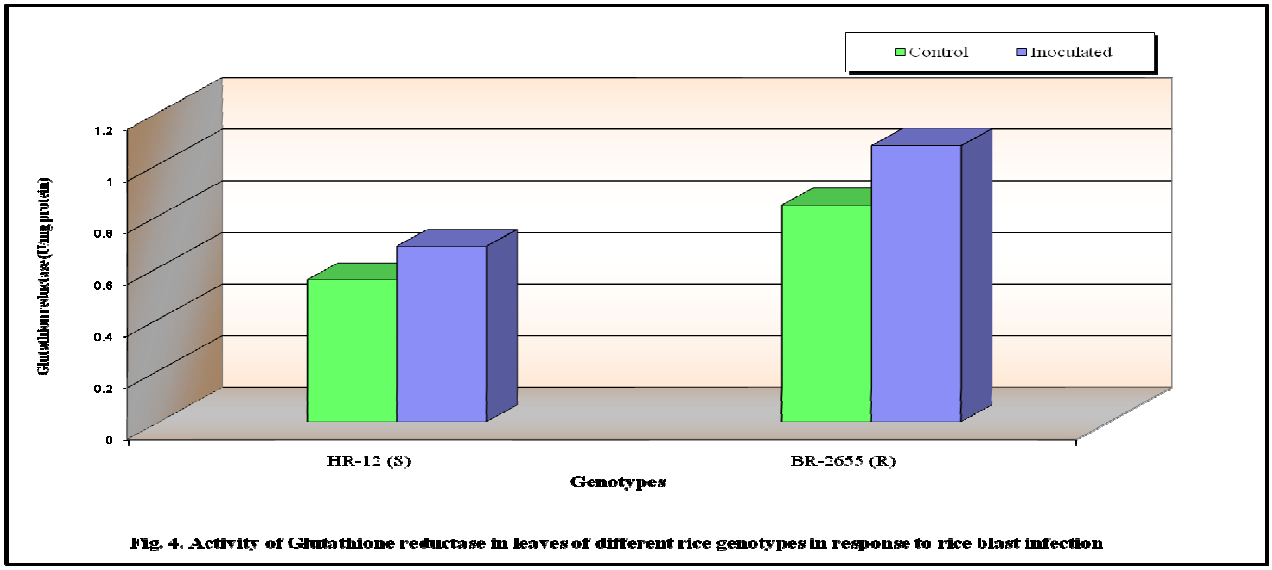

Figure 4: Activity of Glutathione Reductase in Leaves of Different Rice Genotypes in Response to Rice Blast Infection

Table 5: Activity of Phenylalanine Ammonia Lyase in Leaves of Different Rice Genotypes in Response to Rice Blast Infection

Genotype	Phenylalanine ammonia lyase (U/mg protein)			
	Control	Inoculated	% Increase	Mean
HR-12 (S)	1.14	2.05	79.8	1.59
BR-2655 (R)	**1.36**	**3.43**	152.2	2.39
Grand mean	1.12	2.74	116	1.99
	S.Em±		**CD @ 5%**	
Factor G	0.011		0.032	
Factor T	0.011		0.032	
G × T	0.015		0.045	

G: Genotype T: Treatment, R: Resistant genotype, S: Susceptible genotype.

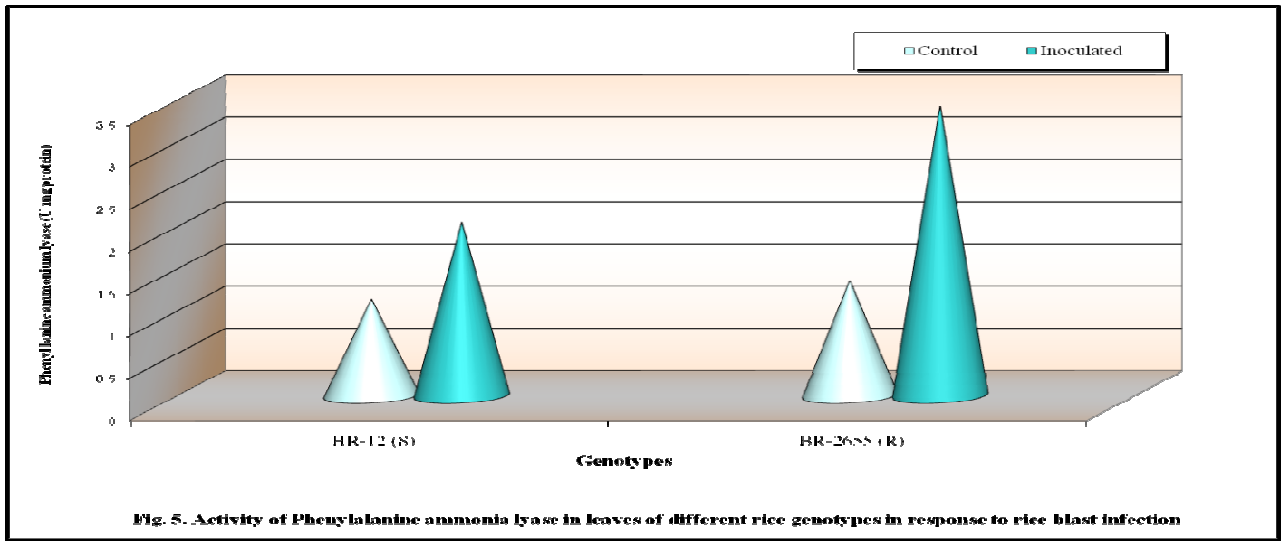

Figure:5 Activity of Phenylalanine Ammonia Lyase in Leaves of Different Rice Genotypes in Response to Rice Blast Infection

Table 6: Activity of Nitrate Reductase in Leaves of Different Rice Genotypes in Response to Rice Blast Infection

Genotype	Nitrate reductase (U/mg protein)			
	Control	Inoculated	% Decrease	Mean
HR-12 (S)	1.15	0.55	51.75	0.84
BR-2566 (R)	**2.01**	**0.93**	53.73	1.47
Grand mean	1.575	0.74	52.74	1.157
	S.Em±		**CD @ 5%**	
Factor G	0.011		0.033	
Factor T	0.011		0.033	
G × T	0.016		0.047	

G: Genotype T: Treatment, R: Resistant Genotype, S: Susceptible Genotype.

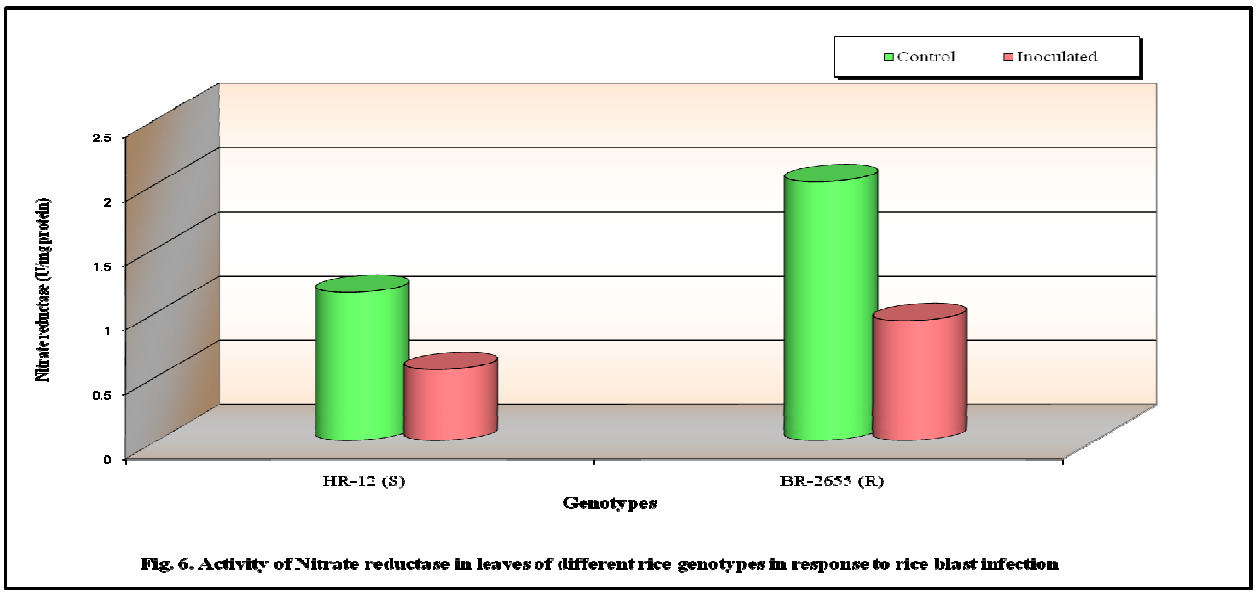

Figure 6: Activity of Nitrate Reductase in Leaves of Different Rice Genotypes in Response to Rice Blast Infection

Table 7: Activity of Nitrite Reductase in Leaves of Different Rice Genotypes in Response to Rice Blast Infection

Genotype	Nitrite reductase (U/mg protein)			
	Control	Inoculated	% Decrease	Mean
HR-12 (S)	0.49	0.27	44.90	0.38
BR-2655 (R)	**0.94**	**0.44**	53.19	0.69
Grand mean	0.71	0.35	54.82	0.53
	S.Em±		**CD @ 5%**	
Factor G	0.09		0.028	
Factor T	0.09		0.028	
G × T	0.013		0.039	

G: Genotype T: Treatment, R: Resistant Genotype, S: Susceptible Genotypes.

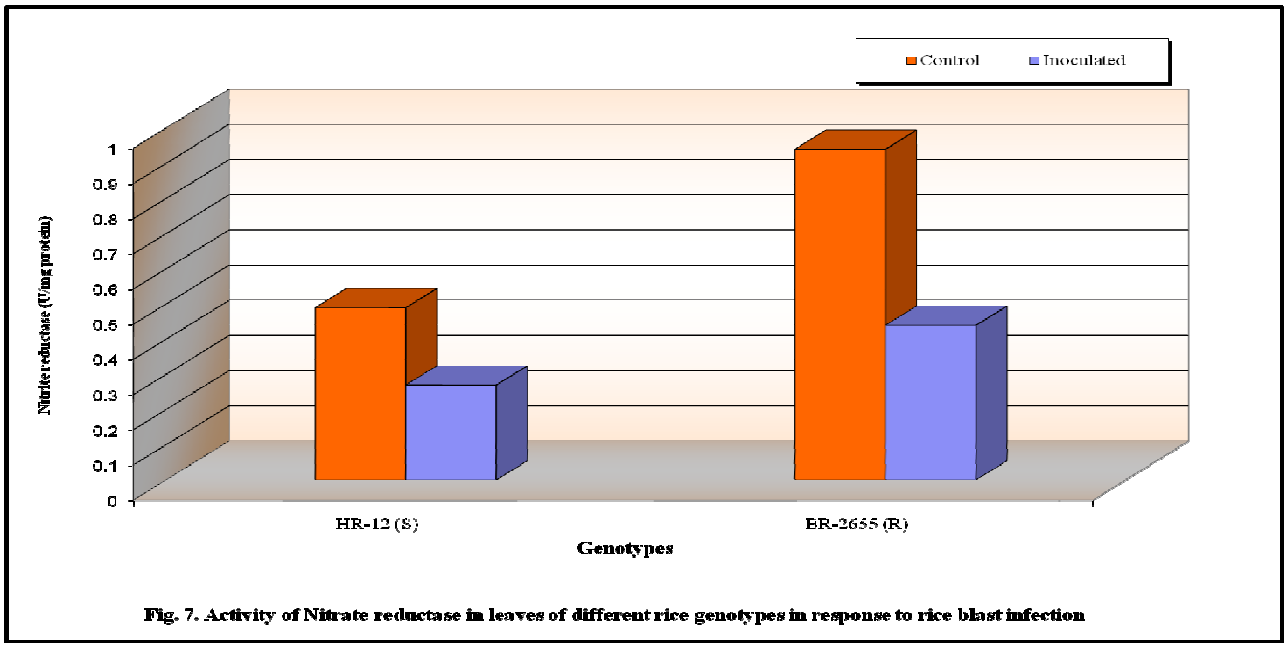

Figure 7: Activity of Nitrite Reductase in Leaves of Different Rice Genotypes in Response to Rice Blast Infection

Table 8: Estimation of Lignin in Leaves of Different Rice Varieties in Response to Rice Blast Infection

Genotype	Lignin (g/100g dry wt)			
	Control	Inoculated	% Increase	Mean
HR-12 (S)	2.93	4.49	53.24	3.71
BR-2655 (R)	**4.91**	**10.33**	110.38	7.6
Grand mean	3.92	7.41	81.81	5.66
	S.Em±		**CD @ 5%**	
Factor G	0.035		0.104	
Factor T	0.035		0.104	
G × T	0.049		0.147	

G: Genotype T: Treatment, R: Resistant Genotype, S: Susceptible Genotype.

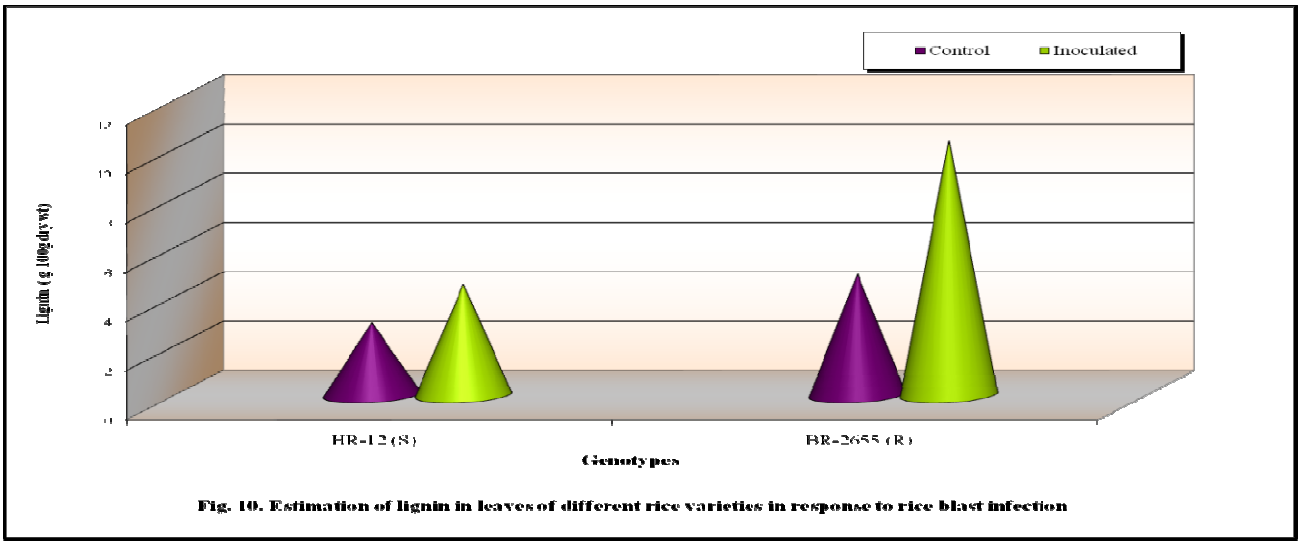

Figure 8: Estimation of Lignin in Leaves of Different Rice Varieties in Response to Rice Blast Infection

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